Null Results in Brief

No Association Between Genetic Polymorphisms of CYP1A1, GSTM1, GSTT1, GSTP1, NAT2, and Nasopharyngeal Carcinoma in Taiwan

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Introduction

NPC[2] is rare in most populations around the world but common in southern China and Southeast Asia. Both genetic and environmental factors are purported to account for the development of NPC. Many Phase I and Phase II enzymes get involved in the metabolism of carcinogens. Some of these enzymes are polymorphic in genotypes that show considerable variation in their activities, which, in turn, determine individual susceptibility to cancer risk (1). No studies to date have examined simultaneously the association between genetic polymorphisms of multiple xenobiotic-metabolizing enzymes and NPC. We have previously reported an association between genetic polymorphism in the CYP2E1 gene and risk of NPC (2). In this study, we examine the association with NPC of genetic polymorphisms of CYP1A1, GSTM1, GSTT1, GSTP1, and NAT2.

Materials and Methods

Study Subjects. The detailed methods of this case-control study have been fully described elsewhere (2). In brief, incident NPC cases were recruited between July 15, 1991, and December 31, 1994, from two teaching hospitals in Taipei, Taiwan. All cases were newly diagnosed, histologically confirmed with NPC, <75 years old of age, and residing in Taipei city or county for at least 6 months. We identified 378 eligible cases. Controls were individually matched with cases on age (within 5 years), sex, and district/township of residence by using the National Household Registration System. A total of 375 cases (99%) and 327 controls (88%) consented to a detailed interview administered by a trained nurse-interviewer. Blood specimens were obtained from 369 cases and 320 controls. In this study, 337 cases (91% of eligible cases) and 317 controls (99% of eligible controls) were included because DNA from the remaining subjects was exhausted by previous testing for other factors.

Laboratory Analysis. DNA was extracted from peripheral blood lymphocytes by standard RNase and proteinase K treatment and phenol-chloroform extraction. Genotypes were analyzed using PCR-based methods as described below.

CYP1A1. The polymorphisms ascribed to *Msp*I in the 3′-flanking region of CYP1A1 were detected by the PCR-RFLP method using the primers described in Kawajiri *et al.* (3). The primer sequences were 5′-CAGTGAAGAGTGTAGCCGCT-3′ and 5′-TAGGAGTCTTGTCTCATGCCT-3′. The CYP1A1 polymorphisms were classified as homozygous for m1/m1, heterozygous for m1/m2, or homozygous for m2/m2 alleles.

GSTM1 and GSTT1. The PCR method for determining GSTM1 and GSTT1 genotypes was the same as that previously reported (4). The primers of GSTM1 were 5′-CTGCCCTACT-TGATTGATGGG-3′ and 5′-CTGGATT- GTAGCGATCATGC-3′. The primers of GSTT1 were 5′-TCACCGGATCATGGCC-AGCA-3′ and 5′-TTCCTTACTGGTCCTCACATCTC-3′.

GSTP1. Determination of genotype at the GSTP1 locus by the PCR-RFLP method was the same as reported by Harries *et al.* (5). The primers were 5′-ACCCCAGGGCTCTATGGGAA-3′ and 5′-TGAGGGCACAAGAAGCCCCT-3′. The GSTP1 polymorphisms were classified as homozygous for GSTP1a (ile105/ile105), heterozygous for GSTP1a/1b (ile105/val105), or homozygous for GSTP1b (val105/val105).

NAT2. The PCR-RFLP method was the same as reported by Hsieh *et al.* (6). The primers were 5′-GGAACAAATTG-GACTTGG-3′ and 5′-TCTAGCATGAATCACTCTGC-3′. Individuals with two mutant alleles including NAT2*5, NAT2*6, NAT2*7 or NAT2*14 were classified as slow acetylators; others were classified as rapid acetylators.

Statistical Analysis. Unconditional logistic regression models were used to estimate the OR and 95% CI to assess the magnitude of the associations between genetic polymorphisms and NPC after controlling for age, sex, and other potential confounders. All statistical significance levels were determined by two-tailed tests. Conditional logistic regression models were not chosen to avoid loss of information from cases and controls without a matched pair. Cases had a slightly higher proportion of Fukkienese ethnicity and lower educational levels than controls. Adjustment for ethnicity and educational level did not alter our findings. This study had at least 80% power to detect a relative risk ≥ 1.5 for each of the genes evaluated.

Results

Frequency distributions of CYP1A1, GSTM1, GSTT1, GSTP1, and NAT2 genotypes and their association with NPC risk are shown in Table 1. No associations with NPC risk were observed for genetic polymorphisms of GSTM1, GSTT1, GSTP1, and NAT2. We further stratified by anti-EBV antibodies, oc-

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[2] The abbreviations used are: NPC, nasopharyngeal carcinoma; CYP, cytochrome P450; GST, glutathione *S*-transferase; RFLP, restriction fragment length polymorphism; OR, odds ratio; CI, confidence interval.

Table 1 ORs for developing NPC for genetic polymorphisms of CYP1A1, GSTM1, GSTT1, GSTP1, and NAT2 after adjustment for age, sex, ethnicity, education level, and exposure to cigarette smoking

Genetic polymorphisms	Cases No. (%)	Controls No. (%)	OR (95% CI)
CYP1A1			
m1/m1	74 (43.0)	83 (38.1)	1.0
m1/m2	75 (43.6)	96 (44.0)	1.2 (0.7–1.8)
m2/m2	23 (13.4)	39 (17.9)	1.4 (0.8–2.6)
GSTM1			
Non-null	141 (44.9)	168 (49.9)	1.0
Null	173 (55.1)	169 (50.1)	0.8 (0.6–1.1)
GSTT1			
Non-null	156 (49.4)	162 (48.2)	1.0
Null	160 (50.6)	174 (51.8)	1.0 (0.8–1.4)
GSTP1			
1a/1a	183 (69.3)	232 (71.8)	1.0
1a/1b	75 (28.4)	86 (26.6)	1.0 (0.6–1.4)
1b/1b	6 (2.3)	5 (1.6)	0.7 (0.2–2.3)
NAT2			
Slow	39 (14.0)	54 (16.6)	1.0
Fast	240 (86.0)	271 (83.4)	0.8 (0.5–1.2)

cupational exposure to wood dust and formaldehyde, and dietary nitrosamine intake; no significant associations of the genotypes examined with NPC risk were noted (data not shown). The frequencies of the CYP1A1 m1 allele among cases and controls were 0.65 and 0.6, respectively. The GSTP1a allele frequencies were 0.69 among cases and 0.85 among controls.

Discussion

We previously reported a 2.6-fold (95% CI = 1.2–5.7) increased risk of NPC among individuals homozygous for a variant form of CYP2E1 detected by *Rsa*I digestion (the c2 allele). This motivated us to evaluate genotypes of other Phase I and II metabolizing enzymes. No association with risk of NPC was noted in our study, however, when genotypes of CYP1A1, GSTM1, GSTT1, GSTP1, and NAT2 were evaluated. In one previous study among 83 NPC cases and 114 controls (7), the GSTM1 null genotype was associated with a marginally significant 1.9-fold (95% CI = 1.0–3.3) increase in NPC risk. Discrepancies with respect to risk associated with the GSTM1 null genotype between this study and that by Nazar-Stewart *et al.* (7) could be attributable to chance or to true differences in the two populations studied. Most subjects in the study by Nazar-Stewart *et al.* (7) were Caucasian, and the predominant histological type of NPC was squamous cell carcinoma. In contrast, in our study, all participants were of Chinese descent and the majority of NPC cases were nonkeratinizing or undifferentiated carcinomas.

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